

AMENDMENTS TO THE SPECIFICATION

Please amend the specification at the paragraph beginning at page 1, line 22, as follows:

In some embodiments, the microfluidic device includes: an input port for receiving a particle-containing liquidic sample, a retention member in communication with the input port and configured to spatially separate particles of the particle-containing liquidic sample from a first portion of the liquid of the ~~particle-containing~~ particle-containing fluidic sample, and a pressure actuator configured to recombine at least some of the separated particles with a subset of the first portion of the liquid separated from the particles.

Please amend the specification at the paragraph beginning at page 2, line 11, as follows:

In some embodiments, a device for concentrating particles of a ~~particle-containing~~ particle-containing fluid includes: a first substrate and a second substrate. The first and second substrates define between them at least a portion of a microfluidic network and a chamber. The microfluidic network includes a first end and a second end. The first end is configured to receive a sample including a particle-containing fluid. The second end of the microfluidic network is in fluidic communication with the chamber. The device also includes a manually actuated member operatively associated with the chamber and configured, upon actuation, to increase a volume thereof, so that a pressure within the chamber decreases drawing fluid toward the second end of the microfluidic network.

Please amend the specification at the paragraph beginning at page 2, line 20, as follows:

In some embodiments, a device for concentrating particles of a particle-containing fluid includes a first substrate and a second substrate. The first and second substrates define between themselves at least a portion of a microfluidic network. The microfluidic network includes a filter configured to allow passage of fluid and to obstruct passage of particles that have a minimum dimension greater than a predetermined value with a source of vacuum in fluidic communication with the filter.

Please amend the specification at the paragraph beginning at page 4, line 30, as follows:

In another embodiment of the present invention, a microfluidic device includes a typically planar substrate including one or more thermally actuated elements. A first side of the substrate includes elements of a microfluidic network, such as a channel and a side channel that intersects the channel. A second, opposed side of the substrate includes a chamber connected to the channel via the side channel. An amount of TRS is disposed in the side channel intermediate the channel and the chamber. Increasing a gas pressure within the chamber may move the TRS into the channel thereby sealing the channel. Advantageously, the chamber and various other elements of the microfluidic network ~~are~~, are located on opposite sides of the substrate thereby allowing more efficient use of the space available on the first side of the substrate.

Please amend the specification at the paragraph beginning at page 5, line 26, as follows:

The valves can include an amount of temperature responsive substance, e.g., wax, to prevent evaporation of the liquid ~~component~~ component.

Please amend the specification at the paragraph beginning at page 7, line 1, as follows:

FIGS. 6c and 6d illustrate the introduction of sample material to the microfluidic device of FIG. 6a, more sample material having been introduced in FIG. 6d than in FIG. 6c.

Please delete the paragraph beginning at page 7, line 2 of the specification.

Please amend the specification at the paragraph beginning at page 7, line 18, as follows

The present invention relates to microfluidic systems and devices and methods for manipulating and processing materials, such as samples and reagents. Microfluidic devices generally include a substrate that defines one or more microfluidic networks, each ~~including~~ including one or more channels, process modules, and actuators. Samples and reagents are manipulated within the microfluidic network(s). The modules and actuators of the networks are typically thermally actuated. For example, a process module can include a reaction chamber that is heated by a heat

source. An actuator may include a chamber that is heated to generate a pressure or a vacuum to move material within the network.

Please amend the specification at the paragraph beginning at page 8, line 12, as follows:

Referring to FIG. 1, an exemplary microfluidic network 110 of a microfluidic device has a sample input module 150 and reagent input module 152 to allow sample and reagent materials, respectively, to be input to ~~device~~ network 110. Generally, one or both of input modules 150, 152 are configured to allow automatic material input using a computer controlled laboratory robot. Network 110 may also include output ports configured to allow withdrawal or output of processed sample from or by microfluidic network 110.

Please amend the specification at the paragraph beginning at page 9, line 17, as follows:

~~Amplification process module~~ PCR-Detection Module 162 receives DNA released from sample particles and reagents and detects minute quantities of DNA therein. In general, process module 162 is configured to amplify the DNA such as by PCR. Detection is typically spectroscopic, as by fluorescence. In some embodiments, the presence and/or abundance of DNA is detected electrochemically.

Please amend the specification at the paragraph beginning at page 9, line 22, as follows:

PCR-Detection module 162 typically includes more than one amplification/detection chamber. One chamber generally receives and detects (with optional amplification) DNA released from sample particles. Another chamber typically receives and detects (with optional amplification) control DNA, which may be used to indicate whether network 110 is functioning properly. Other modules of network 110, e.g., reagent and mixing modules ~~152, 166~~ 152, 166 are configured to accommodate the presence of more than one amplification/detection chamber.

Please amend the specification at the paragraph beginning at page 10, line 8, as follows:

First actuator 168 of network 110 moves material downstream from enrichment module 156 to lysing module 158. Upon completion of processing within lysing module 158, a second actuator

170 moves material downstream to DNA clean-up module 160. Subsequently, actuator 170 or an additional actuator moves cleaned-up DNA to mixing module 166, where the material mixes with a reagent moved by actuator 172. Finally, actuator 172, or another actuator, moves the mixed material to PCR-detection module 162.

Please amend the specification at the paragraph beginning at page 12, line 1, as follows:

Valve 68 is configured in a normally open state in which valve 68 allows passage of material, e.g., gas, along a channel 86 between chamber 66 and port 70. In a closed state, valve 68 obstructs passage of material between chamber 66 and port 70. Valve 68 typically includes a chamber 84 and a mass ~~[[80]]~~ of thermally responsive substance (TRS) 80. In the closed state, the TRS obstructs passage of material whereas in the open state, the TRS is dispersed or withdrawn from the channel to allow passage of material therealong.

Please amend the specification at the paragraph beginning at page 12, line 7, as follows:

Whether for a gate or a valve, the obstructing mass of TRS can have a volume of 250 nl or less, 125 nl or less, 75 nl or less, 50 nl or less, ~~[[25,]]~~ 25 nl or less, 10 nl or less, 2.5 nl or less, 1 nl or less, e.g., 750 ~~pico-liters~~ picoliters or less. In some embodiments of a gate or valve, some or all of the TRS passes downstream upon opening the gate or valve. For example, the TRS may pass downstream along the same channel as sample previously obstructed by the TRS. In some embodiments, the TRS melts and coats walls of the channel downstream from the position occupied by the TRS in the closed state. The walls may be at least partially coated for several mm downstream. In some embodiments, the TRS disperses and passes downstream as particles too small to obstruct the channel. Exemplary gates and valves including a mass of TRS are disclosed in U.S. Pat. No. 6,575,188, issued Jun. 10, 2003, which patent is incorporated herein by reference.

Please amend the specification at the paragraph beginning at page 12, line 25, as follows:

Raising a temperature within chamber 84 increases a pressure therein. When the temperature within chamber 84 is raised and the temperature of TRS 80 is also raised, the pressure within

chamber 84 moves TRS 80 into channel 86 connecting port 70 and chamber 66 thereby obstructing the passage of material, e.g., gas, along channel 86. Substrate 76 includes a heater 82 configured to be in thermal contact with both chamber 84 and TRS 80 when substrates 76 and substrate layer 71 are mated. Actuating heater 82 raises both the temperature within chamber 84 and the temperature of TRS 80 to the second temperature.

Please amend the specification at the paragraph beginning at page 14, line 27, as follows:

Gate 88 has a normally closed state to obstruct passage of material between enrichment region 56 and downstream portions of microfluidic network 51. Gate 88 has an open state in which material may pass from enrichment region 56 to downstream portions of network 51. Gate 88 may be a thermally actuated gate including a mass ~~[[90]]~~ of TRS 90 and actuated by a heat source 92 of substrate 76.

Please amend the specification at the paragraph beginning at page 15, line 1, as follows:

Valve 85 has a normally open state in which material may pass between upstream portions of microfluidic network ~~[[56]]~~ 51 and enrichment region 56. Valve 85 has a closed state, which obstructs material from passing between enrichment region 56 and upstream regions of microfluidic network ~~[[56]]~~ 51. Valve 85 may be a thermally actuated valve including a mass ~~[[89]]~~ of TRS 85 and a chamber 87. Substrate 76 includes a heat source 93 configured to actuate valve 85 as discussed for valve 68.

Please amend the specification at the paragraph beginning at page 15, line 7, as follows:

Enrichment region 56 of device 50 may be operated as follows. A particle containing fluid sample is introduced to network 51, e.g., via port 54, such as by using a syringe or other sample introduction device. The amount of sample introduced may be at least, e.g., 250 microliters, at least 500 microliters, or at least ~~[[1000]]~~ 1,000 microliters. The amount of fluid, e.g., liquid, introduced may be, e.g., less than 10,000 microliters, less than 5,000 microliters, or less than 2,500 microliters. Enrichment region 56 is typically configured so that (with downstream gate

[[90]] 88 closed) fluid entering device 50 must pass through retention member 94 to exit the enrichment region. The particle-containing fluidic sample passes along channel 58 into enrichment region 56.

Please amend the specification at the paragraph beginning at page 17, line 20, as follows:

Typically enrichment ratios, i.e., the volume concentration of particles in the enriched fluid relative to the volume concentration of particles in the introduced fluid, are at least 5, at least 10, at least 25, at least 50 or at least 100. The enriched fluidic sample may be withdrawn from network 51 or subjected to further processing and or analysis therein.

Please amend the specification at the paragraph beginning at page 18, line 9, as follows:

Enrichment region 202, which may be configured as enrichment region 56, receives sample material introduced via port 204 and prepares an enriched sample enriched in a desired particle. Enrichment region 202 includes a retention member 94, a retention member support [[202]] 236, which may be configured as support 96, and a reservoir 234, which may be configured as reservoir 98.

Please amend the specification at the paragraph beginning at page 19, line 9, as follows:

Retention member 94 allows fluid of the sample material to exit enrichment region 202 but retains particles, such as by allowing the passage of fluid but limiting or preventing the passage of the particles as described above. The fluid is expelled through ~~filter~~ retention member 94 and into reservoir 234, which may be sealed as for reservoir 98. Particles of the sample are retained within enrichment region 202 as described above.

Please amend the specification at the paragraph beginning at page 21, line 20, as follows:

As an alternative or in combination with valve 608, device 600 can include a 1-way valve configured to allow sample to enter channel 605 and pass downstream but configured to limit or prevent material, e.g., gas, from passing upstream from ~~from~~ chamber 699 and exiting device

600 via port 654. An exemplary valve is a duckbill valve available from Minivalve International, Oldenzaal, The Netherlands. Such a valve can be located at port 654, e.g., in combination with fitting 655, or disposed along channel 605.

Please amend the specification at the paragraph beginning at page 22, line 1, as follows:

Network 601 includes a passage 635 that connects output port 611 and channel 609. The passage 635 includes a gate 637 to selectively obstruct or allow passage between channel 609 and output port 611. Gate ~~635~~ 637 may have features of gate 216 or other gates (or valves) discussed herein. Gate ~~635~~ 637 is generally configured to have a normally closed state that obstructs passage of material between channel 609 and output port 611.

Please amend the specification at the paragraph beginning at page 23, line 1, as follows:

Device 600 may be operated as follows. A particle-containing sample is introduced to device 600, such as by using a sample introduction device, e.g., a syringe ~~697~~ 696, mated with fitting 655 of input port 654. With valve 608 in the open state and gate 616 in the closed state, sample material passes along channel 605 into enrichment region 602. Pressure created by the sample introduction device drives fluid of the sample through retention member 694 and into chamber 699 of reservoir 698. As discussed above, the entry of fluid into chamber 699 increases the pressure therein. Retention member 694 retains particles of the sample within cavity 691 of enrichment region 602.

Please amend the specification at the paragraph beginning at page 25, line 7, as follows:

Channel 702, upstream of enrichment region 756, includes a valve 708 to selectively obstruct passage of material between input port 754 and enrichment region 756. Valve 708 may have features of valve 208 or other valves (or gates) discussed herein. Valve 708 ~~[[is]]~~ has a normally open state that allows material to pass along channel 702.

Please amend the specification at the paragraph beginning at page 26, line 11, as follows:

Once a sufficient amount of sample material has been introduced, the enrichment region may be sealed to prevent pressure created within chamber 799 from being vented or driving material out of enrichment region 756. For example, valve 708 may be actuated to the closed state to prevent passage of material between input port 754 and enrichment region 756 along channel 702. With valve 708 and gates ~~716, 725~~ 716, 725 in the closed state, device 700 maintains the pressure within chamber 799.

Please amend the specification at the paragraph beginning at page 26, line 31, as follows:

Typically, pressure within chamber 799 also drives the enriched particle-containing sample toward downstream portions of network 701. In some embodiments, a volume of the enriched particle-containing sample driven downstream is determined by a volume of a downstream portion of network 701. For example, with gates ~~725, 759~~ 725, 759 closed and upon actuating gate 716, pressure within chamber 799 drives at least a portion of the enriched particle sample along channel 711 and into channel 723 beyond intersection 713. Enriched sample is driven along channel 723 until a downstream terminus of enriched sample reaches vent 755 inhibiting further movement of the sample. The volume of the enriched sample is substantially determined by a volume of channel 723 intermediate vent 755 and gate 759.

Please amend the specification at the paragraph beginning at page 27, line 15, as follows:

Chamber 799 of device 700 may include one or more additional output ports configured to allow pressure within chamber 799 to be used to manipulate and/or move sample, reagent, or other materials elsewhere within network 701. For example, outlet 717 communicates with channel 733 which itself intersects with channel 709 upstream of lysing region 158. A gate 735 selectively obstructs or allows passage of material between outlet 717 and channel 733. A gate 737 ~~selective~~ selectively obstructs or allows passage of material between channels 709 and 733. Upon preparation of a lysed sample, gates ~~735, 737~~ 735, 737 are opened whereupon pressure from chamber 799 moves the lysed sample downstream of lysing chamber 158.

Please amend the specification at the paragraph beginning at page 27, line 31, as follows:

Sample material passes along channel 308 to enrichment region 302. The fluid travels into the concentration region (including a circular filter whose center is typically free and whose edge 309 is secured to the chip) and through the filter leaving the cells or other particles of interest behind at an internal surface of the filter. The waste fluid, may pool on top of the device, and can be discarded, assuming a thin meniscus of liquid remains on the top of the filter to prevent drying and to provide a reservoir from which to backflow. Once the cells are trapped by the filter, the user actuates the valve 310, thus obstructing the passage of material between port 304 and enrichment region 302. Then the tape is removed, and an external device, e.g., a pipette or syringe, is used to backflow ~~the~~ some of the liquid of the sample back through the filter to re-entrain the cells. Typically, less than 25%, less than 10%, less than 5%, less than 2.5%, or less than 1% of the fluid introduced with the particles re-entrains the particles.

Please amend the specification at the paragraph beginning at page 28, line 19, as follows:

A thermopneumatic actuator 1014 generates a gas pressure sufficient to move material, e.g., a lysed sample, downstream from lysing region 1006 and into channel 1018. Actuator 1014 typically operates by generating an upstream pressure increase but device ~~1001~~ 1000 can be configured with an actuator that provides a downstream pressure decrease, e.g., a partial vacuum, to move material downstream from lysing region 1006. A gate 1071 selectively obstructs or allows passage of material between actuator 1014 ~~and~~ and lysing chamber 1006.

Please amend the specification at the paragraph beginning at page 29, line 1, as follows:

A thermopneumatic actuator ~~1022~~ 1007 generates a gas pressure sufficient to move material, e.g., an amount of reagent, downstream from reagent metering chamber 1024 and into channel 1018. Actuator ~~1022~~ 1007 typically operates by generating an upstream pressure increase but ~~device~~ network 1001 can be configured with an actuator that provides a downstream pressure decrease, e.g., a partial vacuum, to move material downstream from reagent metering region

1024. A gate 1073 selectively obstructs or allows passage of material between actuator ~~1022~~ 1007 and reagent metering region 1024.

Please amend the specification at the paragraph beginning at page 29, line 8, as follows:

With gates ~~1022,1042~~ 1022, 1042 in the open state, downstream portion 1020 of lysing region 1006 and downstream portion 1028 of reagent metering chamber 1024 lead to an intersection 1019, which is the upstream terminus of a channel 1018. The channel 1018 leads to a reaction chamber 1048 having an upstream terminus defined by a valve 1050 and a downstream terminus defined by a valve 1052. Valves 1050, 1052 can be closed to prevent material from exiting reaction chamber 1048. A vent 1054 allows degassing, debubbling of material passing along channel 1018 into chamber 1048. A vent 1055 prevents pressure buildup from preventing material from entering chamber 1048.

Please amend the specification at the paragraph beginning at page 29, line 16, as follows:

Gates and valves of network 1001 are typically thermally actuated and may have features of other valves and gates discussed herein. For example, valve 1011 includes a mass of TRS 1059 and a pressure chamber 1057. Increasing a temperature of ~~[[of]]~~ TRS 1059 and a pressure within chamber 1057 drives TRS 1059 into channel thereby obstructing the channel. Gate 1022 includes a mass of TRS 1061 that obstructs passage of material from lysing region 1006 to intersection 1019. Raising a temperature of TRS 1061 allows upstream pressure (or a downstream partial vacuum) to move material from lysing region into intersection 1019 and channel 1018.

Please amend the specification at the paragraph beginning at page 29, line 24, as follows:

Vents of network 1001 typically include a porous hydrophobic membrane as discussed for vents of other devices herein. The vents allow gas to escape network 1001 ~~put~~ but inhibit or prevent liquid from escaping.

Please amend the specification at the paragraph beginning at page 29, line 27, as follows:

Device 1000 is typically configured to receive a ~~cell-containing~~ cell-containing sample, lyse the cells to release intracellular material, combine the intracellular material with reagents, e.g., reagents suitable for PCR amplification and detection, deliver the combined reagents and intracellular material to the reaction chamber 1048, amplify DNA present in the intracellular material, and detect the presence or absence of a particular type of cell, e.g., group B strept, based upon the detected DNA.

Please amend the specification at the paragraph beginning at page 30, line 14, as follows:

Reagent materials may be introduced to network 1001 via port 1032. Waste channel 1038 and waste port 1039 cooperate with reagent metering region 1024 to deliver an amount of reagent materials and position the reagent materials in the same way that waste channel 1008 and waste port 1009 cooperate with lysing chamber 1006 to deliver an amount of sample and position the sample. Reagent materials may also be stored on the device during manufacture as discussed elsewhere herein.

Please amend the specification at the paragraph beginning at page 30, line 20, as follows:

Within the sample introduced and present within lysing chamber 1006, valves ~~1011, 1005~~ 1011, 1005 are closed. Closure of valves ~~1011, 1005~~ 1011, 1005 isolates sample within lysing chamber 1006 from the atmosphere surrounding device 1000. By isolate, it is meant that sample material present within lysing chamber 1006 may be heated by an amount sufficient to lyse cells therein without significant evaporation of liquid accompanying the cells. In one embodiment, for example, material within lysing chamber 1006 may be heated to as much as 93.degree. C., 95.degree. C., or 97.degree. C., for as long as 1 minute, 2 minutes, 3 minutes, or even 5 minutes without substantial loss of the liquid within the lysing chamber. In some embodiments, less than 20%, less than 10%, less than 5%, or less than 2.5% of the liquid present in the lysing chamber is lost. In some embodiments, lysing chamber 1006, like lysing chambers of other lysing modules

disclosed herein, has a volume of less than 5 microliters, less than 1 microliter, less than 500 nl, less than 200 nl, less than 100 nl, less than 50 nl, e.g., less than 10 nl.

Please amend the specification at the paragraph beginning at page 30, line 32, as follows:

As discussed above, valves ~~1011, 1005~~ 1011, 1055 typically include a mass of TRS, e.g., wax such as parafin, that operates to obstruct or allow passage of material. In the closed state, it is the TRS that obstructs gas and heated liquid from exiting lysing chamber 1006 (or reaction chamber 1048 for valves ~~1050, 1052~~ 1050, 1052). In some embodiments, the obstructing mass of TRS can have a volume of 250 nl or less, 125 nl or less, 75 nl or less, 50 nl or less, 25, nl or less, 10 nl or less, 2.5 nl or less, 1 nl or less, e.g., 750 pico liters or less. Some or all of the TRS can pass downstream as discussed above.

Please amend the specification at the paragraph beginning at page 31, line 6, as follows:

Sample in ~~lysing~~ lysing chamber 1006 is locally heated for a specified amount of time at a specific temperature to break open the target cells to release intracellular contents which include genetic material such as DNA. Heating lysing chamber 1006 is typically localized to prevent perturbation of other components of device 1000. For example, if gates ~~1071, 1073~~ 1071, 1073 are thermally actuated gates, heat used to lyse cells within lysing chamber 1006 generally does not cause premature opening of these gates (or other gates of the device).

Please amend the specification at the paragraph beginning at page 31, line 12, as follows:

Turning to FIG. 9c, upon lysing cells of sample within chamber 1006, gates ~~1071, 1073, 1022, 1042~~ 1071, 1073, 1022, 1042 are opened. An open state of gate 1071 provides communication between actuator 1014 and an upstream portion of lysing chamber 1006 adjacent upstream opening 1012. An open state of gate 1022 provides communication between sample present within lysing chamber 1006 and downstream portions of the microfluidic network. An open state of gate 1073 provides communication between actuator ~~1022~~ 1007 and an upstream portion of reagent metering region 1024. An open state of gate 1042 provides communication

between reagent present within metering region 1024 and downstream portions of the microfluidic network.

Please amend the specification at the paragraph beginning at page 31, line 20, as follows:

Pressure source 1022 in actuator 1014 is activated causing a pressure difference between the upstream and downstream portions of sample present within lysing chamber 1006. Typically, an upstream pressure increases relative to a downstream pressure, causing an amount of the sample to move downstream, for example to a downstream channel 1018 (FIG. 16e). ~~Pressure source~~ 1022 Actuator 1007 is activated causing a pressure difference between the upstream and downstream portions of ~~reagent~~ reagent within region 1024. Typically, an upstream pressure increases relative to a downstream pressure, causing an amount of the sample to move downstream, for example to a downstream channel 1018, where the reagent mixes with the lysed contents of the sample.

Please amend the specification at the paragraph beginning at page 31, line 28, as follows:

The volume of sample moved downstream from the lysing chamber 1006 is typically known. In the embodiment shown, for example, the volume is determined by the volume of lysing chamber 1006 between upstream and downstream portions 1012, 1020 thereof. Valves ~~1057, 1005~~ 1057, 1005 may cooperate in preparation of a known amount of sample by closing alternative passages into which material present in lysing chamber 1006 might flow upon actuation of ~~pressure source~~ actuator 1014.

Please amend the specification at the paragraph beginning at page 32, line 1, as follows:

Referring back to FIG. 9a, device 1000 combines a known amount of reagent with sample material, preferably with sample 1016 including released cellular contents. The volume of reagent combined with the sample is determined by a volume of network 1001 intermediate an outlet 1075 of actuator ~~1022~~ 1007 and gate 1042. Sample and reagent material ~~moves~~ move along channel 1018 into reaction chamber 1048. Once reagents and sample material are present

within chamber 1048, valves ~~1050,1052~~ 1050, 1052 are closed. The sample reagent mixture within chamber 1048 is typically subjected to one or more heating and cooling steps, such as to amplify polynucleotides present in the sample reagent mixture.

Please amend the specification at the paragraph beginning at page 32, line 15, as follows:

In the closed state, valves ~~1050,1052~~ 1050, 1052 limit or prevent evaporation of the sample reagent mixture during reaction, for example, by isolating the sample reagent mixture from the surrounding atmosphere. In some embodiments, the sample reagent mixture may be heated to between about 90 °C and about 99.75 °C, for example between about 92 °C and about 98 °C, for example about 97 °C, for at least about 2 minutes, for example between about 3 minutes and about 10 minutes with a loss of no more than about 10 percent by weight, for example, no more than about 5 percent, or no more than about 2.5 percent of the sample reagent mixture.

Please amend the specification at the paragraph beginning at page 32, line 29, as follows:

In some embodiments, heat sources, e.g., resistive heaters, are located external to device 1000 but in ~~thermal~~ thermal communication with the outer surface of the second layer. In another embodiment, heat sources are integrally formed with device 1000, for example, within the first, injection molded layer. Exemplary placement and operation of heat sources is discussed below and elsewhere herein.

Please amend the specification at the paragraph beginning at page 33, line 1, as follows:

Device 1000 may also include a third layer, which is preferably disposed adjacent a surface of the first layer that abuts the second layer. Thus, the second and third layers may sandwich the first layer therebetween. The third layer may contact a surface of the first layer that includes only a subset, if any, of the components of the microfluidic network 1001. In some embodiments, however, access ports and vents provide passage between the microfluidic network 1001 and the opposed surface of the first layer. For example, access ports ~~1002,1032~~ 1002, 1032 may be configured to allow sample material to be introduced through the third layer and into the

microfluidic network. The ports can be configured to mate with a sample introduction device, such as a syringe.

Please amend the specification at the paragraph beginning at page 33, line 10, as follows:

Waste ports ~~1009, 1039~~ 1009, 1039 can extend through the first and third layers to a reservoir into which excess sample and reagents introduced to the microfluidic network may be received and contained from spillage.

Please amend the specification at the paragraph beginning at page 33, line 13, as follows:

Vents ~~1004, 1034~~ 1004, 1034 and other vents of device 1000 can extend through the first and third layers. Typically, the vents include a hydrophobic filter that allows passage of gases but inhibits passage of cells or aqueous liquids.

Please amend the specification at the paragraph beginning at page 33, line 21, as follows:

As discussed elsewhere herein, microfluidic devices include gates and valves to ~~selectively~~ selectively obstruct or allow passage of material within microfluidic networks. For example, gates and/or valves can prevent the evaporation of liquid from a sample subjected to heating such as for lysing cells or amplifying DNA. As discussed herein, gates typically include a mass of TRS, which, in the closed state, obstructs passage of material along a channel. Upon opening the gate, at least a portion of the TRS typically enters a downstream channel of the device. Valves typically operate by introducing a mass of TRS into an open channel to obstruct the channel. An exemplary device for use as a fluid control element, e.g., a gate or valve, is discussed below.

Please amend the specification at the paragraph beginning at page 34, line 1, as follows:

Device 500 can be operated as a normally closed device, e.g., a gate, which opens upon actuation to allow passage between upstream and downstream portions 504, 506. Device 500 can also be operated as a normally open device, e.g., a valve, which closes upon actuation to obstruct passage between upstream and downstream portions 504, 506. Thus, device 500 combines

features of both gates and valves, as discussed herein, and may be used in the place of any gate or valve of any device disclosed herein.

Please amend the specification at the paragraph beginning at page 35, line 15, as follows:

In some embodiments, however, device 500 includes a single source of heat that both raises a pressure within chamber 514 and raises the temperature of TRS 510. Using a single heat source reduces the number of electrical ~~connects~~ connections required to operate device 500.

Please amend the specification at the paragraph beginning at page 35, line 18, as follows:

Typically, channels ~~502, 508~~, 502, 508 hole 516, and chamber 514 are fabricated by injection molding layer 522. Layers 520 and 522 are typically mated to layer 522 by lamination. Layer 524 typically covers the entire open portion of chamber 514 and is preferably sufficiently rigid (or is provided with additional support elements) to withstand flexing during pressurization of chamber 514.

Please amend the specification at the paragraph beginning at page 35, line 27, as follows:

Device 500 may be opened by actuating heater 532 and applying pressure within channel 502 to move TRS present in the gate region. Device 500 may be closed again by actuating heater 530 to pressurize chamber 514 and heat TRS 510 present in hole 516 and side channel ~~510~~ 508 to the second more mobile temperature. The pressure within chamber 514 moves TRS 510 into gate region 507. Heater 532 may also be actuated to close device 500.

Please amend the specification at the paragraph beginning at page 36, line 24, as follows:

Vacuum generator 404 includes a chamber 408 defined between first and second gaskets ~~412, 413~~ 412, 413 of a plunger 410. Chamber 408 is in communication with network 401 via a channel 414. Plunger 410 and gasket 412 slide within substrate 409 expanding the size of chamber 408 between a surface 417 of gasket 412 and gasket 413. Plunger 410 and gasket 412 typically slide along a plunger axis, which is substantially parallel to a plane of network 401 and substrate 409.

Vacuum generator 404 thus generates a reduced pressure within chamber 408 to manipulate material, e.g., sample material and/or reagent material, therein. In a preferred embodiment, the reduced pressure assists the introduction of sample material to device 400. In another embodiment, the reduced pressure assists the preparation of an enriched sample.

Please amend the specification at the paragraph beginning at page 37, line 1, as follows:

As plunger 410 is depressed (moved further into substrate 409), the size of chamber 408 increases. Preferably, channel 414 provides the only passage for gas to enter chamber 408. Thus, depressing plunger 410 creates a pressure differential between chamber 404 408 and network 401 urging material downstream within network 401 and toward chamber 404 408. The actuation of plunger 410 to create the at least partial vacuum typically decreases a dimension d, of device 400. Chamber 408 and gaskets 412, 413 typically prevent leakage of material that might be drawn into chamber 408.

Please amend the specification at the paragraph beginning at page 40, line 20, as follows:

A microfluidic device including an epoxy-based substrate defining a 500 nl lysing chamber covered by a glass coverslip was prepared. About 500 nl of the GBS of Example 1 was loaded into the lysing chamber. The input port was sealed with an adhesive polymer. ~~Microfluidic device was lysed for two minutes at~~ The chip was placed on a heater and lysed at 97 °C for 2 min. The sample was retrieved by pipette and the volume of sample ~~were~~ was brought up to 10 . μ l with TE buffer (pH 8.0). About 2 μ l of this diluted sample was subjected to PCR. The experiment was repeated several times. The PCR amplification results demonstrate that a time of 2 min was sufficient to lyse the cells.